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# Detection of fresh-cut produce processing residues on food contact surface materials using hyperspectral imaging

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Abstract To reduce the risk of foodborne-illness, produce processors currently clean and sanitize food contact surfaces daily before production starts. Current methods to verify the efficacy of cleaning procedures include visual inspection and direct surface sampling using ATP bioluminescence assays and culturing methods. To assess the possibility of augmenting these existing verification methods, this study investigated the potential to use imaging techniques to detect fresh-cut produce residues. A laboratory hyperspectral system was used to image produce residues obtained from a commercial processing plant, cantaloupe and honeydew residues generated in-house, and selected cleaning and sanitizing agents. Test materials were dispensed onto stainless steel and high density polyethylene coupons. The coupons were selected to represent common surfaces used in production facilities. Analysis of VIS/NIR hyperspectral reflectance and fluorescence images showed that the cleaning and sanitizing agents were essentially undetectable; thus, demonstrating that presence of these substances would not result in false-positives. In contrast, produce residues in microgram quantities showed fluorescence peaks encompassing the regions from 480 to 560 nm and from 670 to 690 nm. However, auto-fluorescence responses of high density polyethylene at shorter wavelengths were found to obscure the 480 to 560 nm peaks for some residues. These results suggest that fluorescence imaging techniques can be used to enhance surface hygiene inspection in produce processing plants and, given the immediate availability of imaging results, to help optimize routine cleaning procedures.

Keywords Hyperspectral imaging · Fluorescence imaging · Food safety · Produce · Sanitation

## Introduction

In the United States an estimated 48 million cases of foodborne illness occur annually with 29.4 % of these illnesses deriving from produce and associated products [1, 2]. From 1996 to 2006, 72 foodborne illness outbreaks were associated with fresh produce consumption with 18 of these connected to fresh-cut produce [3]. Contamination on food processing surfaces is one potential source of pathogen transfer to finished products. Improper cleaning and sanitizing of equipment including pumps, tanks, and containers used to handle raw food products has resulted in numerous incidents of cross-contamination of food products [4–7]. In addition, produce debris or residues remaining on surfaces after cleaning can harbor and support the growth of microorganisms that can cause spoilage or foodborne illness, or lead to biofilm development [8]. To reduce the risk of foodborne illness, produce processors clean and sanitize food contact surfaces before production starts as part of a General Hygiene Plan (GHP). An important component of these GHPs includes methods for validating that the cleaning procedures result in food contact surfaces that are hygienic.

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Current surface hygiene verification methods include culturing techniques, ATP bioluminescence assays, and visual inspection with the naked eye. Culturing techniques and ATP bioluminescence assays are used to sample small surface areas based on random, representative, selective, or convenience sampling plans [9]. However, these assays require disposable reagents and can only test a small portion of any surface area [10]. In addition, culturing tests require a wait time of 24–48 h and ATP tests require a wait time of 1–3 min before results are known. Visual inspection with the naked eye represents an inexpensive method for companies to survey large surface areas in real-time, but is limited by human factors including attention span and visual acuity.

A camera-based imaging system has the potential to enhance visual inspection by improving sensitivity and, with the addition of processing capabilities, support development of specific detection algorithms. To ascertain the feasibility of such an imaging system, a laboratory hyperspectral imaging system will be used to image common produce residues found in fresh-cut processing plants against backgrounds of materials commonly used for construction of cutting boards, tables, and other processing equipment. Acquisitions of images of cleaning and sanitizing agents will allow determination of the potential for false-positives from these sources.

While it is possible to acquire both VIS/NIR reflectance and fluorescence imaging using a laboratory hyperspectral imaging system, literature results suggest that fluorescence imaging will offer greater detection sensitivity. Produce contains organic compounds that have been demonstrated to fluoresce, with the fluorescence related to highly conjugated non-aromatic, aromatic, and heterocyclic structures [11]. Chlorophyll-a, a key element in plant photosynthesis, fluoresces strongly between 670 and 680 nm [12]. Kok [13] reported that chlorophyll-a in plants fluoresces at 680 and 740 nm bands. Chappelle et al. [14] found fluorescence bands with maxima at 420, 440, 490, and 525 nm for vitamin K, reduced nicotinamide adenine dinucleotide (NADPH), beta-carotene, and riboflavin, respectively. In addition, lignins and other phenolics found in plant material emit blue fluorescence under ultraviolet light excitation [15–17]. Furthermore, Whitehead et al. [18] have demonstrated that fluorescence imaging at select wavebands can detect processing residues that can harbor microorganisms that are not detectable with ATP assays.

## Materials and methods

A laboratory hyperspectral system was used to acquire reflectance and fluorescence images of residues obtained from a local fresh-cut processing plant and generated in-house using produce purchased at local markets, as well as images of cleaning and sanitizing agents obtained from local processing plants. These image data were used to investigate the potential for development of a portable imaging system for real-time use in produce processing plants.

### Hyperspectral imaging system

The laboratory line-scan hyperspectral imaging system developed by the USDA Agricultural Research Service, Environmental Microbial and Food Safety Laboratory has been well described (Fig. 1, [19]). The systems includes an electron-multiplying charge-coupled device (EMCCD) camera (MegaLuca R, ANDOR Technology, South Windsor, CT), an imaging spectrograph (VNIR Concentric Imaging Spectrograph, Headwall Photonics, Fitchburg, MA), a C-mount lens (F1.9 35 mm compact lens, Schneider Optics, Hauppauge, NY), and two fiber optic bundles to randomly arranged rectilinear fiber bundles (Fiber-Lite A-240P, Dolan-Jenner Industries, Lawrence, MA) for reflectance imaging and two UV-A lamps (XX-15N, Spectronics Corp., Westbury, NY) for fluorescent excitation. A high pass optical filter (Kodak Wratten 4A) was used to dampen responses at wavelengths below 480 nm.

Software developed in-house using Microsoft Visual Basic (version 6.0, Microsoft, Seattle, WA) was used to acquire registered VIS/NIR reflectance and fluorescence images. Vertical pixels (spectral) were binned by four, which resulted in 76 distinct spectral bands between 460.4 and 699.9 nm at * 3.2 nm intervals. Horizontal pixels were

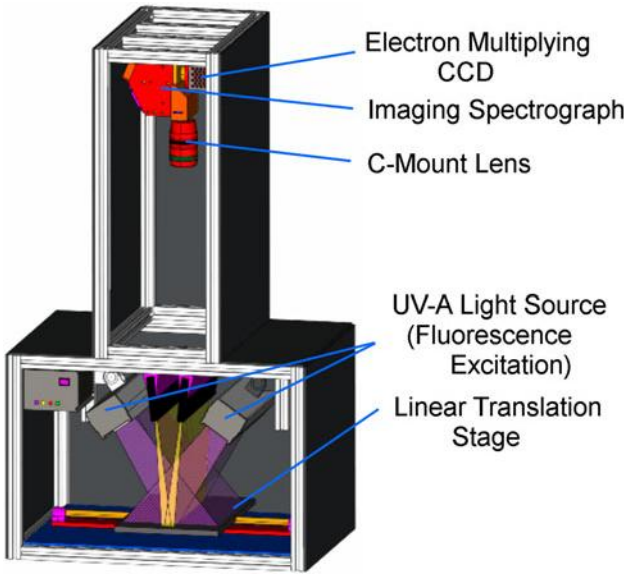


Fig. 1 Laboratory based hyperspectral fluorescence imaging system

binned by two, which resulted in a pixel count of 502 and a spatial resolution of * 0.25 mm per pixel. In order to produce images with matching horizontal and vertical spatial resolution, the translation table was increment in 0.25 mm steps. Gain and exposure were set to 0 and 0.001 s, and 15 and 0.1 s for reflectance and fluorescence imaging, respectively. Images were corrected for dark current and, for reflectance images, white balance prior to analyses.

## Samples for testing

Processing residues from cutting boards for fresh-cut cantaloupe, honeydew, pineapple, salsa (mostly tomatoes), strawberry, and watermelon were collected at a local fresh-cut processing plant by using a squeegee to concentrate materials on processing surfaces, and then sampling the concentrated materials using one mL disposable pipettes. Residues were transferred to 50 mL polypropylene test tubes and held on ice until being frozen and then thawed for experimental use. Western cantaloupe and honeydew residues were also generated in-house using produce purchased at local markets. To replicate procedures used at the processing plant, the melons were submerged in a 100 ppm free chlorine solution and cut on a high-density polyethylene cutting board. The top and bottom portion of each melon was removed with medial cuts and discarded. The remaining rind was removed with vertical cuts and discarded. After cutting the melon in half with a longitudinal slice, the seeds and pulp within the seed cavity were manually removed with a scoop. The remaining hemispheres of the melon were cut into 2–3 cm slices and chopped into 1–2 cm wide pieces [20]. The resulting melon pieces were removed from the board by hand and the remaining material collected using the same procedures that were used in-plant.

All produce residues were characterized by measuring their pH, soluble solids content, and total solids content. The pH was measured three times with a pH probe (AB15, Fisher Scientific, Pittsburgh, PA) and averaged. (°Brix) was measured with a hand refractometer (AO 10431, Scientific Instruments, Keene, NH) two times and averaged. The total solids content was measured using a standardized drying method (AOAC 44.1.03 B).

Cleaning and sanitation solutions were obtained from two local fresh-cut processing facilities (Table 1). The solutions were diluted using distilled water to the manufacturer's recommended concentrations for use.

## Sample presentation

The produce residues and cleaning solutions were dispensed onto 10.24 9 10.24 cm stainless steel (SS) and high density polyethylene (HDPE) coupons. The coupons were initially virgin material. Prior to use, or re-use, coupons were washed with dish soap (Dawn, Proctor & Gamble, Cincinnati, OH), rinsed once with SaniHol 70 (Decon Laboratories, Inc., King of Prussia, PA), once with distilled water, and then dried.

Serial-dilutions of residues from a selected in-house cantaloupe and honeydew were prepared at ratios of 1–10, 1–20, 1–40, and 1–80 in distilled water to facilitate sensitivity testing. Three replicate 100 l L drops of raw or diluted materials were dispensed onto coupons in a row parallel to the image scanning direction to minimize potential effects of uneven lighting. Coupons were imaged immediately after application of materials and again after the materials had dried.

## Image analyses

In-house software developed using Microsoft Windows Visual Basic (Version 6.0) was used to calculate the average intensity by spectra of a rectangular area encompassing 54–66 pixels near the center of each test droplet (Fig. 2). Unfortunately, it was found that mercury emission peaks of the UV excitation light source produced artificially responses peaks between 533.9 to 559.4 nm and

Table 1 Cleaners and sanitizers selected for imaging

| Cleaner or sanitizer | Manufacturer | Description |
|---|---|---|
| CleanEdge 3022 | CleanEdge, Baltimore, MD | Sodium hydroxide and sodium hypochlorite based cleaner |
| CleanEdge 6911 | CleanEdge, Baltimore, MD | Industrial degreaser |
| Sodium hypochlorite | Clorox, Oakland, CA | Sodium hypochlorite sanitizer |
| CleanEdge 3510 | CleanEdge, Baltimore, MD | Alkyl dimethyl, octyl decyl, dioctyl dimethyl, and didecyl dimethyl ammonium chloride sanitizer |
| Tsunami 200 | Ecolab, St. Paul, MN | Acid based sanitizer |
| Sterilex Ultra-Kleen Liquid Cleaner | Sterilex, Owings Mills, MD | Hydrogen peroxide, tetrasodium EDTA, sodium carbonate, and potassium carbonate |
| Alpet D2 | Best Sanitizers, Inc., Penn Valley, CA | Isopropyl alcohol and glycerol based sanitizer |

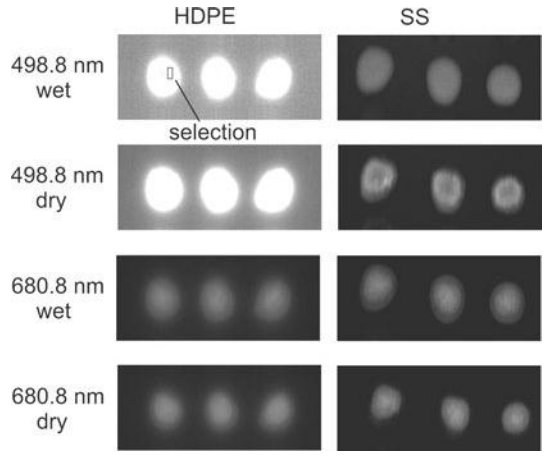


Fig. 2 Examples of fluorescence images of residues from a single cantaloupe both wet and after drying on HDPE and SS at the two peak spectral wavelengths. The selection rectangle shows a region of interest used to determine the average response intensity that was subsequently used for calculating the spectral response of the cantaloupe

between 572.2 to 585.0 nm. As these artifacts were found in otherwise featureless regions of the spectra, the effected regions were replaced by interpolated second-order least-squares estimates. The adjusted spectra for each of the three replicate test droplets were then averaged and graphed using Excel (2007, Microsoft, Seattle, WA). These graphs were visually inspected to identify spectral regions where relative fluorescent intensity (RFI) peaks occurred or where spectral intensities differed from those of control spectra from untreated areas on coupons.

## Results

Spectra for fluorescence images showed similar responses trends for all produce residues tested. However, responses by coupon type were different due to auto-fluorescence responses of HDPE, particularly at shorter wavelengths. Even in the red region, responses for materials on SS coupons were lower than corresponding responses for HDPE coupons. The enhanced responses in the red region for HDPE coupons are presumably due to fluorescence energy transfer from the blue region. Results for cleaning agents indicated that these substances do not fluoresce with UV excitation. Data for dried residues are not presented as these results were very similar to the corresponding data for wet residues; however, spectral amplitudes were slightly increased, presumably due to decreased water content and the consequential reduction in water quenching. Data for reflectance images are not presented as these images were not found to be useful for detection of produce residues.

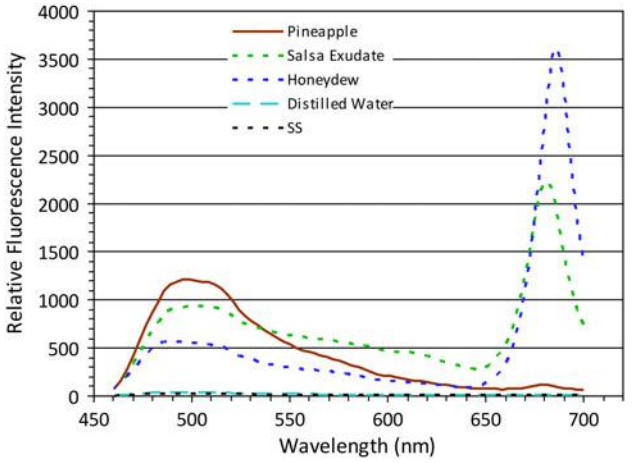


Fig. 3 Fluorescence spectra of various fresh-cut produce processing residues on SS

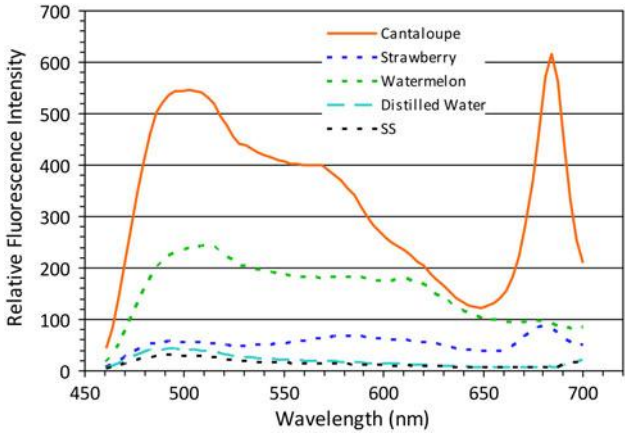


Fig. 4 Fluorescence spectra of additional fresh-cut produce processing residues on SS. Note the reduced y-axis scale compared to Fig. 3

### Produce residue samples acquired in processing plants

On SS coupons, produce residues generally showed a broad fluorescence response centered at around 498.8 nm, and a sharper peak starting at 658.4 nm and peaking at around 680 nm (Figs. 3, 4). Between about 500 and 660 nm, a number of residues showed a broad, low amplitude, peak. The peak wavelength of this broad peak varied. Residues could be differentiated from coupon backgrounds at essentially all wavelengths tested. For HDPE coupons, results were complicated by a broad auto-fluorescence response with a peak around 500 nm and a long linear decrease from 500 to around 650 nm (Figs. 5, 6). For watermelons and strawberries, the magnitude of the responses exceeded the responses of untreated HDPE and of distilled water drops only for the peaks at around 680 nm. Differences of responses across residues were not related to pH, soluble solids content, or total solids content (Table 2).

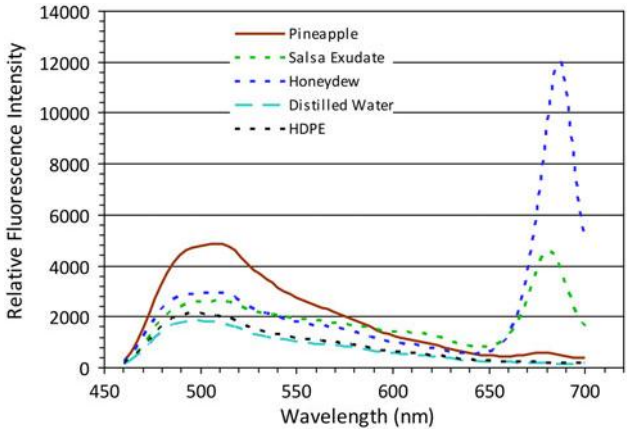


Fig. 5 Fluorescence spectra of various fresh-cut produce residues on HDPE

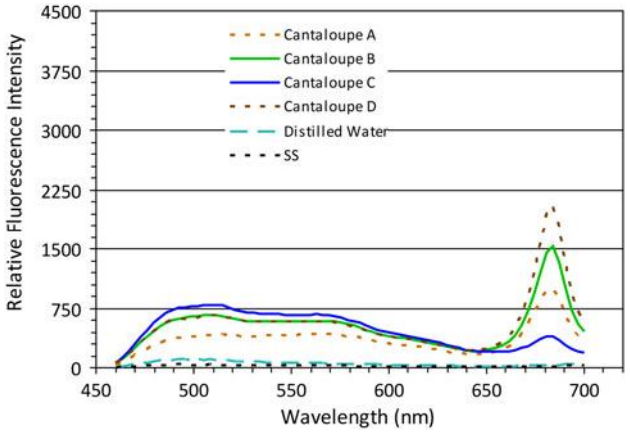


Fig. 7 Fluorescence spectra of fresh-cut cantaloupe residues on SS

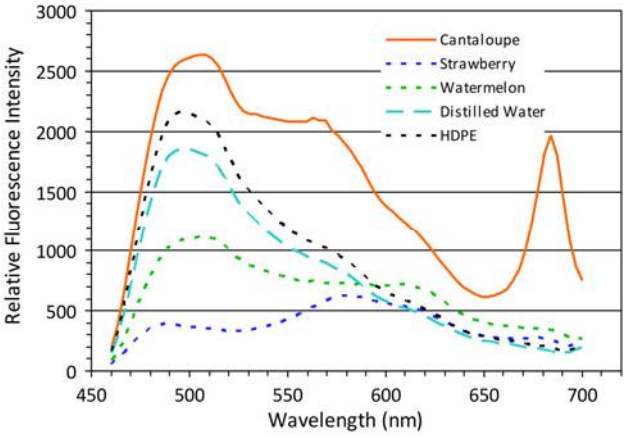


Fig. 6 Fluorescence spectra of additional fresh-cut produce processing residues on HDPE. Note the reduced y-axis scale compared to Fig. 4

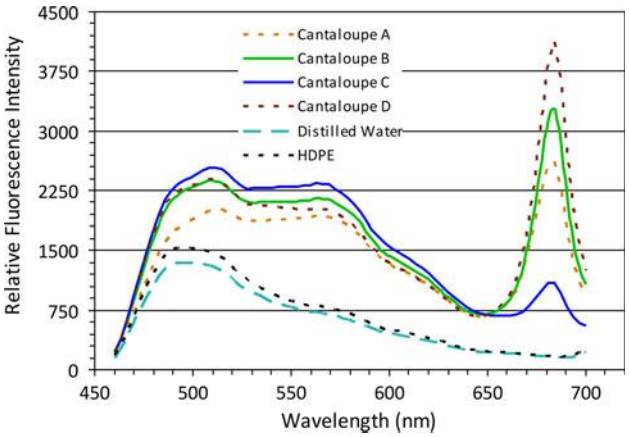


Fig. 8 Fluorescence spectra of fresh-cut cantaloupe residues on HDPE

Table 2 The pH, soluble solids (°Brix) and total solids content of processing residues in Figs. 3, 4, 5, 6

| Solution | pH | Soluble solids (°Brix) | Total solids (%) |
|---|---|---|---|
| Honeydew | 6.05 | 7.75 | 8.34 |
| Cantaloupe | 6.78 | 4.25 | 8.95 |
| Watermelon | 5.89 | 8.25 | 8.64 |
| Salsa Exudate | 3.87 | 8.50 | 4.49 |
| Pineapple | 3.64 | 11.25 | 11.54 |
| Strawberry | 3.65 | 8.00 | 8.26 |

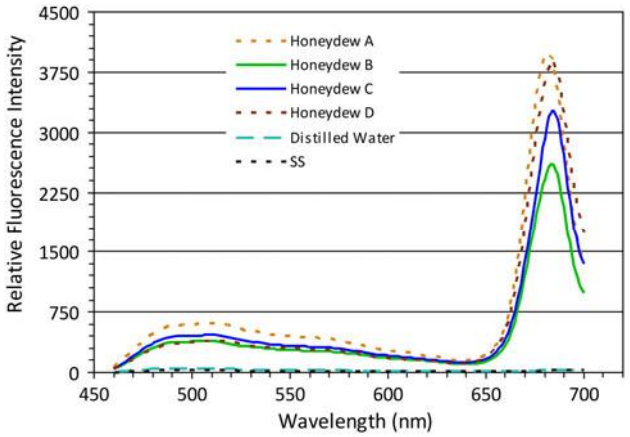


Fig. 9 Fluorescence spectra of fresh-cut honeydew residues on SS

## Produce residue samples generated in-house

The fluorescence spectra of both cantaloupe and honeydew were similar in shape, with a broad peak in the blue region and a sharper peak at around 680 nm (Figs. 7, 8, 9, 10). The effects of the HDPE auto-fluorescence were evident as exaggerated responses at lower wavelengths. Spectra for SS and distilled water on SS steel showed no distinct peaks or troughs. Spectra for HDPE showed peaks at 498.8 nm

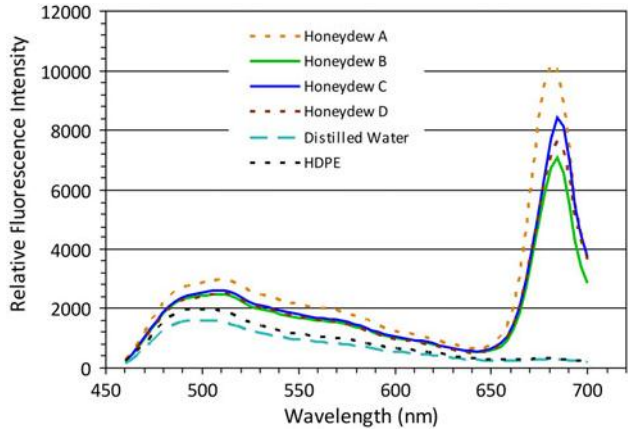


Fig. 10 Fluorescence spectra of fresh-cut honeydew residues on HDPE

and a trough at 693.6 nm. Distilled water on HDPE had a lower RFI response than HDPE alone, presumably due to quenching.

The response profiles for cantaloupe and honeydew residues were similar to those seen for corresponding residues obtained from the produce processing plant (Figs. 5, 6). The average spectra for all cantaloupes exceeded the spectra of the coupons and of water placed on the coupons at all wavelengths for SS and from 460.4 to 699.9 nm for HDPE. RFI peaks for the canaloupe occurred near 511.3, 562.6, and 684.0 nm with troughs between 645.7 and 655.2 nm for both SS and HDPE. The spectra of honeydew residues exceeded the background material and distilled water on the background material at all measured wavelengths for both SS and HDPE. RFI peaks occurred near 511.3 and 684.0 nm with troughs near 642.5 nm. Differences in amplitudes of spectra among either type of melon could not be correlated to pH, nor total or soluble solids content (Tables 3, 4).

The fluorescence spectra of dilutions of residues from Cantaloup B residue were similar in shape to spectra for raw cantaloupes, with a monotonic decrease in amplitude with dilution (Figs. 11, 12). For SS, the amplitude of spectra for all dilutions exceeded the spectra for the coupon at all wavelengths. For HDPE, the amplitude of spectra above about 650 nm for all dilutions exceeded the amplitude of the spectra for HDPE; however, only the spectra for the 1:10 dilution exceeded the spectra for HDPE below

Table 3 The pH, soluble solids (°Brix), and total solids content of four fresh-cut cantaloupe

| Cantaloupe | pH | Soluble solids (°Brix) | Total solids (%) |
|---|---|---|---|
| A | 6.90 | 7.25 | 10.06 |
| B | 7.86 | 10.25 | 9.98 |
| C | 7.47 | 11.50 | 10.95 |
| D | 7.57 | 9.25 | 8.84 |

Table 4 The pH, soluble solids (°Brix), and total solids content of four fresh-cut honeydew melon

| Honeydew | pH | Soluble solids (°Brix) | Total solids (%) |
|---|---|---|---|
| A | 6.39 | 9.63 | 9.33 |
| B | 6.49 | 10.00 | 9.54 |
| C | 6.98 | 9.00 | 8.52 |
| D | 6.92 | 8.25 | 7.99 |

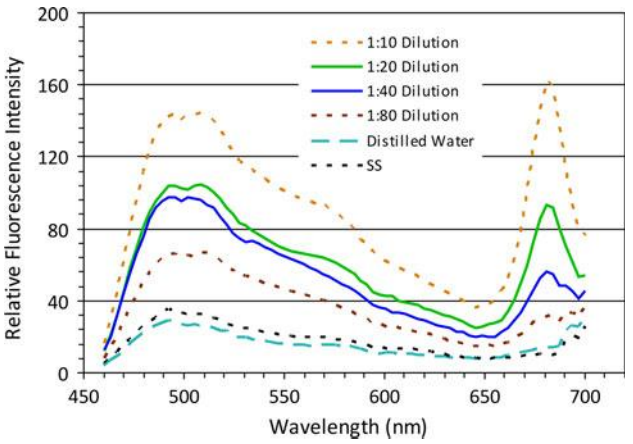


Fig. 11 Fluorescence spectra of dilutions of a fresh-cut cantaloupe residues on SS

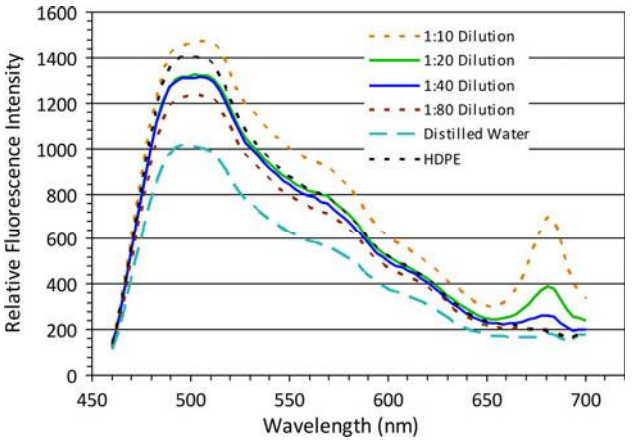


Fig. 12 Fluorescence spectra of dilutions of a fresh-cut cantaloupe residues on HDPE

650 nm. Results (not shown) for dilutions of a honeydew residue showed similar effects.

## Cleaners and sanitizers

The fluorescence emission spectra of cleaners and sanitizing agents on SS and HDPE are in Figs. 13 and 14, respectively. For SS, the spectra of these materials were essentially indistinguishable from the spectra of SS. For

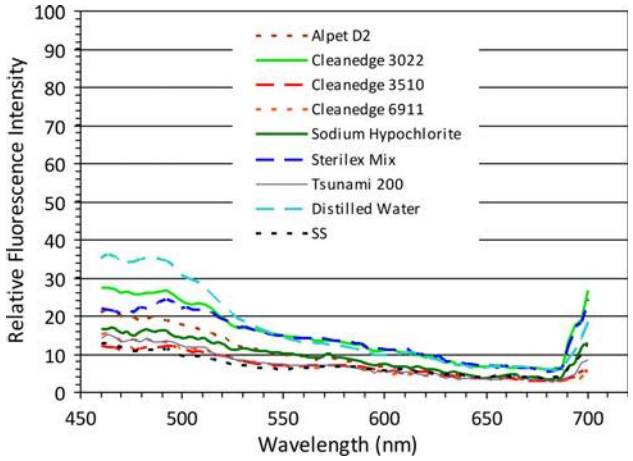


Fig. 13 Fluorescence spectra of cleaners and sanitizers on SS

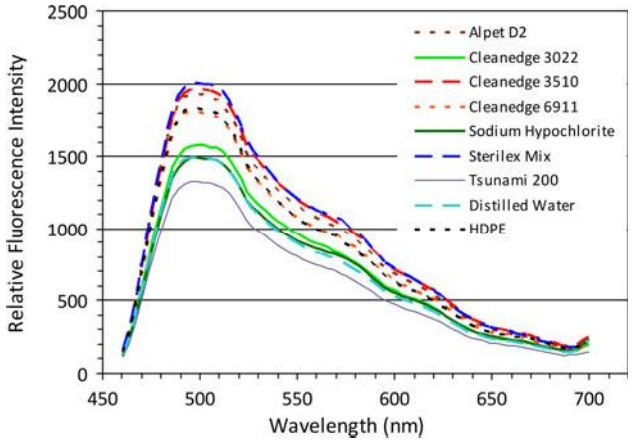


Fig. 14 Fluorescence spectra of cleaners and sanitizers on HDPE

HDPE, spectra were hard to interpret due to the auto-fluorescence response of HDPE; however, spectra were essentially identical for all materials on HDPE at longer wavelengths.

## Discussion

In produce plants, the substances likely to be on processing surfaces following cleaning and sanitation procedures are residues from processed produce and sanitizing agents. The produce residues are not supposed to be there, while it is often desirable to leave a coating of cleaning and sanitizing agents. In order to be able to use imaging techniques to survey the hygiene of processing surfaces subsequent to cleaning and sanitizing procedures, it is critical that the imaging techniques can detect small quantities of residues and do not detect cleaning and sanitizing agents. For detection of residues, it is not really important if the measured intensity of residues at a given wavelength is greater or less than that of the background material on which it resides. For monochrome images, if the intensity is greater, a residue will appear as a whiter area or spot. If the intensity is lower, it will appear as a darker area or spot. The results of this study clearly demonstrate that it is possible to differentiate small quantities of residues from background materials common used for processing surfaces in produce processing plants at essentially all wavelengths tested. Assuming a uniform density of 1 for residues, a solids content of 10 % for the 100 l L drops corresponds to 10 l g of material. The pixel area used to estimate spectra, * 50 pixels, represents about 10 % of the droplet area. Thus, a single pixel corresponds to a 0.25 9 0.25 mm area and 20 ng of material. This extreme sensitivity using a laboratory measurement system suggests that detection of produce residues using a less sensitive portable system is feasible. Results for cleaning and sanitizing agents indicate that these type substances will not produce false positives at these detection limits. If the ability to detect cleaning or sanitizing solutions became an issue, a food-grade fluorescent dye could be added to the solutions.

The results of this study demonstrate the potential for using fluorescence imaging to detect produce residues that may be present in processing plants after completion of cleaning and sanitizing procedures. One additional question is whether it might be possible to determine the original source of a detected residue. Given the lack of differentiating spectral features among tested materials, this is not a realistic goal. However, the inability to indentify the source of a residue does not really impact the potential usefulness of fluorescence imaging as the essential goal of a survey would be to detect anything that is not supposed to be present. One approach might be to bias detection parameters to yield greater sensitivity at the potential cost of more false positives. Because results from the survey would be immediately available, it would be possible to re-clean any detect problem site. Human judgment could be used to determine if a site that remained "contaminated" following re-cleaning was a false-positive.

There are some of additional factors that should be considered before efforts are made to translate current results into a test instrument to be used in a processing facility. The first consideration is the cost and complexity of the survey device. A device with a single excitation source and an inexpensive camera with a single bandpass filter in front of the lens would probably be the least expensive device. On the other end of the cost spectrum would be a device with multiple excitation sources, multiple filters or a tunable filter, a sensitive camera with low noise, and image processing capabilities. Theses added capabilities would allow the use of sophisticated detection algorithms that consider spatial variability in images and/or

differences among images acquired at two or more wavelengths; e.g., ratios of images acquired at two different wavelengths. Such techniques have been demonstrated to greatly enhance detection capabilities [21]. Another consideration is lighting in processing plants. The laboratory measurements were made in the dark and it is unrealistic to consider turning-off all lights in a facility during instrumented surveys. The fluorescence responses measured in this study could be obscured by ambient lighting. One way of addressing this dilemma is to consider the lighting source in a plant. Most industrial plants utilize lighting that does not have a uniform spectrum. For example, most of the energy in emissions from fluorescent lights is centered in narrow bands related to mercury excitation and the phosphors used in the lamps. Areas in the visible spectrum that coincide with low output from the lighting would probably be candidate wavelength regions for fluorescence detection of possible contaminants. As the illumination spectrum is dependant on the particular lighting in a plant, including the particular characteristics of the lamps in use, it might be beneficial to measure the lighting characteristics of a given plant and to adjust the detection wavelength or wavelengths appropriately. Another possible solution would be to take images with and without UV excitation, and to look at differential responses. This solution would require image processing capabilities.

The samples in this study showed a broad fluorescence peak in the blue-green and a narrow peak in the red regions of the visible spectrum. One design for a survey instrument would be a unit with two switchable bandpass filters in these two regions. However, given the need to consider ambient lighting conditions, it would be premature to suggest optimal center frequencies or widths of passbands. The influence of the auto-fluorescence of HDPE should also be considered in selecting filter parameters. Given these uncertainties, attempts to use the current data to develop more sophisticated detection algorithms would also be premature. To address these uncertainties, consideration should be given to developing a test device for use in processing plants that includes the ability to examine ambient light intensities and fluorescence responses at multiple wavelengths.

## Conclusion

The results of this study provided encouragement for the use of fluorescence imaging as the basis of a tool for surveying the hygienic status of surfaces in produce processing plants following normal cleaning procedures. Tests of various produce residues demonstrate that fluorescence imaging can be used to detect microgram quantities of materials, and that cleaning and sanitizing solutions will not produce false positives.

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